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ECHITAMINE

BY

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CCXVIII.—*Echitamine.*

By John Augustus Goodson and Thomas Anderson Henry.

The alkaloid echitamine was first prepared by Gorup-Besanez (*Annalen*, 1875, **176**, 88) from the bark of *Alstonia scholaris*, R.Br. (*Echites scholaris*, L.) and was the subject of much controversy between O. Hesse (*Annalen*, 1875, **176**, 326; **178**, 49 (with Jobst); 1880, **203**, 144; *Ber.*, 1880, **13**, 1841) and Harnack (*Arch. exp. Path. Pharm.*, 1877, **7**, 126; *Ber.*, 1878, **11**, 2004; 1880, **13**, 1648) in the course of which it was shown that Harnack's ditaine (derived from "dita bark," a native name for the bark of *A. scholaris*) is identical with echitamine. Hesse's observations on the alkaloid

were largely confirmed by Bacon (*Philippine J. Sci.*, 1906, **1**, 1007). The position arrived at may be summarised thus : *A. scholaris* bark contains at least two alkaloids : (*a*) ditamine, amorphous, isolated by extracting with ether the residue from an alcoholic extract of the bark, rendered alkaline with ammonia, and (*b*) echitamine (ditaine), obtained by extraction of the same residue, rendered alkaline with solution of sodium or potassium hydroxide, with chloroform, after the removal of ditamine. It is clear from this preliminary work that echitamine is a strong base which, unlike ditamine, cannot be liberated from its salts by ammonia, and the separation of the two alkaloids is based on this fact. The first description of echitamine was given by Hesse (*Annalen*, 1880, **203**, 144), who stated that the free base (echitammonium hydroxide) formed glassy prisms, $C_{22}H_{30}O_5N_2,3H_2O$, m. p. 206°, $[\alpha]_D^{15°}$ — 28·8° in alcohol, lost $3H_2O$ at 80° and a fourth molecule of water at 105° in a vacuum, forming a new base, $C_{22}H_{28}O_4N_2$, weaker than the original, but yielding the same hydrochloride, $C_{22}H_{28}O_4N_2,HCl$. Harnack, on the contrary, assigned the formula $C_{22}H_{30}O_4N_2,HCl$ to the hydrochloride.

In addition to *A. scholaris*, several other species of *Alstonia* have been examined. Hesse found in *A. constricta* the amorphous bases porphyrine and chlorogenine (subsequently renamed alstonine) and the crystalline alkaloid, alstonidine (*Annalen*, 1865, Suppl. IV, p. 40; 1880, **205**, 360; *Ber.*, 1878, **11** ,1546. Compare von Muller and Rummel, J., 1879, **35**, 31; Oberlin and Schlagdenhauffen, *J. Pharm.*, 1879, **28**, 576). From a Java species, *A. spectabilis*, R. Brown, Hesse obtained echitamine, ditamine and two other alkaloids, echitenine and alstonamine, the last-named substance being crystalline (*Ber.*, 1878, **11**, 1546; *Annalen*, 1880, **203**, 170), but could find no trace of Scharlec's alstonine (*Genees. Tijd. Ned. Ind.*, 1863, **10**, 209).

Much work has also been done on the sterols and other crystalline, non-nitrogenous substances of Alstonia species. Jobst and Hesse found in *A. scholaris* bark, echikautschin (amorphous); echicerin, $C_{30}H_{48}O_2$, m. p. 157°, $[\alpha]_D$ + 63·75°; echitin, $C_{32}H_{52}O_2$, m. p. 170°, $[\alpha]_D$ + 75·25°; echitein, $C_{42}H_{70}O_2$, m. p. 195°, $[\alpha]_D$ + 85·4°, and echiretin, $C_{35}H_{56}O_2$ (amorphous).

From the dried latex of *Alstonia costulata*, Miq. (*Dyera costulata*, Hook) Sack and Tollens isolated a similar series of sterols (*Ber.*, 1904, **37**, 4110), which Cohen (*Arch. Pharm.*, 1907, **245**, 236) subsequently identified with lupeol, and α- and β-amyrins, and Ultée (*Chem. Weekblad*, 1914, **11**, 456) found the same three constituents in the latex of *A. scholaris*.

The material used in the present investigation consisted of bark

derived from *A. congensis*, Engler. The first sample was collected in the Cameroons by Dr. Lehmann, and for it and the supplies subsequently received from the Cameroons, the Conservator of Forests, Gold Coast, and the Director of Forests, Nigeria, the authors are indebted to the kindness of Dr. Hill, Director of the Royal Botanic Gardens, Kew.

The results of the present investigation confirm Hesse's empirical formula for echitamine, and many of the unexplained points recorded by Hesse and Harnack in the course of their controversy as to the formula of echitamine are accounted for by the fact now established that echitamine is a methyl ester and is hydrolysed with great ease, forming the new base *demethylechitamine*, $C_{21}H_{26}O_4N_2$. It is also shown that echitamine contains a methylimino-group, but whether this has a tertiary or secondary nitrogen is uncertain, as the alkaloid appears to yield a crystalline nitroso-derivative. For the same reason, it is uncertain whether one or two hydroxyl groups are present, as the second acetyl group in the diacetylechitamine now described may indicate the presence of either a hydroxyl group or a secondary nitrogen atom.

Finally, the fact that the parent alkaloid and all the derivatives described give an intense blue colour with Hopkin and Cole's glyoxylic reagent for tryptophan (*Proc. Roy. Soc.*, 1901, **68**, 21) must be taken to mean that echitamine has an indole nucleus, and support for this view is given by the production of an indole-like base, as one product, when echitamine is distilled with alkalis.

A. congensis bark, like other barks of this genus, contains much amorphous alkaloid in addition to echitamine and yields a large quantity of non-nitrogenous products. Of the latter, one substance, a well-crystallised lactone, $C_9H_{14}O_3$, was isolated as a by-product in the purification of echitamine and has been characterised. It is converted by sodium hydroxide into a crystalline sodium salt, $C_9H_{15}O_4Na$, and yields a monoacetyl derivative, so that its formula may be extended thus, $HO\cdot\overline{C_8H_{15}CO\cdot O}$.

For some of the echitamine used in this investigation the authors are indebted to Prof. F. L. Pyman, F.R.S., who isolated it from the bark of another species, *A. Gillettii*, kindly supplied by Fr. Just. Gillet, Curator of the Botanic Gardens, Kisanti, Belgian Congo.

The various Alstonia barks are used in the localities where they occur, as remedies for malaria, but so far no attempt has been made to use echitamine in medicine. The authors are indebted to Dr. J. Trevan of the Wellcome Physiological Research Laboratories for the observation that echitamine hydrochloride is toxic to mice

in doses of 0·3 to 0·5 mg. per 20 g. and that it acts by paralysis of the medulla.

In connexion with the use of the bark in malaria Major Brown of the Wellcome Bureau of Scientific Research found that the hydrochloride was not toxic to protozoa (Glaucoma) at a concentration of 0·03%, but became so at a concentration of 0·0025% in presence of *N*/800-alkali (compare Bacon, *loc. cit.*).

Experimental.

The finely-ground bark (1 kg.) was exhausted with hot alcohol, the concentrated solution poured into warm 1% acetic acid (500 c.c.), and the filtrate from this concentrated to 200 c.c. After agitation with ether (extract A) and chloroform (extract B) in turn to remove wax and other impurities, the liquor was rendered alkaline with sodium hydroxide solution and the liberated alkaloids were dissolved out with chloroform. The residue from the chloroformic solution was dissolved in alcohol, and the liquid rendered slightly acid with hydrochloric acid (10%) and set aside. Echitamine hydrochloride gradually crystallised and was collected, washed with a little alcohol, and recrystallised from water. The yield of crystalline hydrochloride from different samples of *A. congensis* bark varied considerably.

	Gold Coast.	Nigeria.	Cameroons.
Total alkaloid %	0·38 to 0·56	0·11 to 0·12	0·18
Echitamine hydrochloride %	0·18 to 0·34	0·03 to 0·04	0·09

As few data are recorded by previous workers regarding the base and its salts, the opportunity has been taken to characterise these substances.

The hydrochloride obtained was identical with echitamine hydrochloride from *A. scholaris* bark. When crystallised quickly from water, it forms long, anhydrous needles, m. p. 295° (corr.; decomp.), $[\alpha]_D^{15°}$ — 58° (in water; $c = 1\%$) (Found : C, 62·6, 62·9; H, 7·1, 7·1; N, 6·4; Cl, 8·4, 8·5. $C_{22}H_{28}O_4N_2$, HCl requires C, 62·8; H, 6·9; N, 6·9; Cl, 8·4%). When crystallised slowly from water, it forms stumpy prisms, m. p. 292° (corr.; decomp.), $[\alpha]_D^{15°}$ — 54·3° ($c = 1·028$; water) (Found : loss at 100° in a vacuum, 4·79. Calc. for $C_{22}H_{28}O_4N_2$,HCl,H_2O : H_2O, 4·15%). Both forms of the hydrochloride are neutral to litmus. Hesse (*loc. cit.*) records for his hydrochloride $C_{22}H_{28}O_4N_2$,HCl, $[\alpha]_D$ — 57° ($c = 2$; water). The hydrobromide, similarly obtained, forms transparent prisms, m. p. 258° (air-dry; corr.; decomp.), 268° (dry; corr.; decomp.), $[\alpha]_D^{15°}$ — 43·5° for the hydrated salt ($c = 0·804$ in water) (Found : C, 56·4; H, 6·5; Br, 16·9. Calc. for $C_{22}H_{28}O_4N_2$,HBr : C, 56·8; H, 6·3; Br, 17·2%. Loss on drying at 105° in a vacuum, 3·7. Calc. for $C_{22}H_{28}O_4N_2$,HBr,H_2O :

H_2O, 3·7%). Hesse gives the formula $C_{22}H_{28}O_4N_2,HBr,2H_2O$ to his hydrobromide.

The hydriodide, similarly prepared, crystallises from water in long, anhydrous prisms, m. p. 267° (corr.; decomp.) (Found : C, 51·4; H, 5·9; I, 24·0. Calc. for $C_{22}H_{28}O_4N_2,HI$: C, 51·6; H, 5·7; I, 24·8%).

The sulphate is more soluble than the halide salts, separates only after considerable concentration of the neutralised solution, and is best recrystallised by adding alcohol to a strong solution of the salt in water. It forms rosettes of needles, which decompose from 275° onwards; $[\alpha]_D^{15°}$ — 51·6° (c = 2·58 in water) [Found for air-dry salt : loss at 100° in a vacuum, 2·4. $(C_{22}H_{28}O_4N_2)_2,H_2SO_4,H_2O$ requires H_2O, 2·04%. Found in dry salt : H_2SO_4, 11·46. Calc., H_2SO_4, 11·31%].

The *nitrate*, obtained by neutralising the base in chloroform with *N*/10-nitric acid, separates, on cooling the concentrated aqueous solution, in elongated pyramids, $C_{22}H_{28}O_4N_2,HNO_3,2H_2O$, m. p. 127° (air-dry; corr.; decomp.), 176° (dry; corr.), $[\alpha]_D^{15°}$ — 51·4° (air-dry salt in water; c = 1·01) (Found : loss on drying in a vacuum over sulphuric acid, 8·15. Calc., 7·45%. Found in dry salt : C, 58·9; H, 6·8. Calc., C, 59·0; H, 6·5%).

The neutral oxalate, $(C_{22}H_{28}O_4N_2)_2,H_2C_2O_4$, was only obtained as a gelatinous precipitate, although Hesse (*loc. cit.*) obtained it crystalline (Found : C, 63·9; H, 6·7. Calc., C, 64·3; H, 6·8%). When the calculated quantity of oxalic acid is added to a solution of the base in alcohol, the neutral oxalate first formed dissolves and on adding a little water and removing the alcohol by distillation in a vacuum, rosettes of needles of *echitamine hydrogen oxalate*, $C_{22}H_{28}O_4N_2,H_2C_2O_4,2H_2O$, m. p. 238° (corr.; decomp.), separate on cooling (Found : loss on drying at 100° in a vacuum, 6·3. Calc., H_2O, 7·05%. Found in dry salt : C, 61·0; H, 7·0. Calc., C, 60·7; H, 6·4%).

The picrate is precipitated when picric acid solution is added to the hydrochloride dissolved in water. The air-dry substance softens at 50°, decomposes at 98°, and loses in a vacuum at atmospheric temperature over sulphuric acid 41·3%, corresponding to $24H_2O$. On recrystallisation from dilute alcohol it forms minute rosettes of needles, $C_{22}H_{28}O_4N_2,C_6H_2(OH)(NO_2)_3,2H_2O$, which behave like the air-dry substance on heating (Found : loss on drying in a vacuum over sulphuric acid, 5·5. Calc., 5·6%. Found in dry substance : C, 54·6; H, 5·1. Calc., C, 54·8; H, 5·1%).

Determinations of methoxyl and methylimino-groups by the usual methods on the hydrochloride and hydriodide gave the following results. Hydrochloride : Found; MeO, 7·2; NMe, 7·6. Calc.

for MeO, 7·4; NMe, 6·9%. Hydriodide: Found; MeO, 6·0, 6·1; NMe, 6·0. Calc. for MeO, 6·1; NMe, 5·7%.

Diacetylechitamine Hydrochloride.—Echitamine hydrochloride was mixed with a few drops of pyridine and heated at 100° with six parts by weight of acetic anhydride. On cooling, the diacetyl derivative separated in silky masses of needles and more was obtained by concentrating under reduced pressure. After recrystallisation from ethyl acetate the substance had m. p. 271° (corr.; decomp.) [Found in substance dried at 105° in a vacuum: C, 61·7; H, 6·7; Cl, 6·8; MeO, 6·6; acetic acid (by hydrolysis with phosphoric acid), 26·2. $C_{22}H_{26}O_4N_2Ac_2,HCl$ requires C, 61·8; H, 6·6; Cl, 7·0; MeO, 6·2; acetic acid, 23·8%].

Hydrolysis of Echitamine.—The base used in preparing the salts described above was obtained by dissolving the hydrochloride, as isolated (p. 1643), in water, adding sodium hydroxide, and extracting with chloroform. If complete removal of this solvent by distillation is attempted, the base decomposes as already recorded by Hesse (*Annalen*, 1880, **203**, 144), who called the product oxyechitamine and gave it the formula $C_{22}H_{28}O_5N_2$. The change is probably more profound than Hesse supposed, but the substance has not been examined in the course of this work. The solvent can, however, be largely removed by distillation and the residue, dissolved in alcohol, used for the preparation of salts, but it has not been possible to induce the base to crystallise, though Hesse, as already stated, obtained a base which he at one time regarded as a crystalline tetrahydrate, $C_{22}H_{28}O_4N_2,4H_2O$. In the course of attempts to crystallise the base, water was added (after some days) to a solution in alcohol, prepared as described, and on long standing this deposited crystalline material, more of which was slowly obtained by continued dilution with water.

The same substance was produced more rapidly by heating echitamine hydrochloride in sealed tubes at 120° during 6 hours with 0·24*N*-sodium hydroxide solution (20 c.c. per g. of hydrochloride). It can be recrystallised from hot 70% alcohol and then forms prisms or hexagonal plates, m. p. 290° (air-dry; corr.; decomp.) or 268° (dry; corr.; decomp.). It is sparingly soluble in alcohol, chloroform, or water and is neutral to litmus. It forms a *hydrochloride* crystallising in prisms from hot water, m. p. 306° (corr.; decomp.), which is acid to litmus and much less soluble in water (1 in 209, approx.) than echitamine hydrochloride (1 in 76 approx.) (Found for air-dry base: C, 62·2; H, 7·5; loss in a vacuum at 130°, 8·7. $C_{21}H_{26}O_4N_2,2H_2O$ requires C, 62·0; H, 7·4; H_2O, 8·9%. Found for base dried at 130° in a vacuum: C, 68·0, 68·3; H, 7·3, 7·3; N, 7·8. $C_{21}H_{26}O_4N_2$ requires C, 68·1; H, 7·1; N, 7·6%.

Found for hydrochloride, dried at 100° in a vacuum : C, 62·1, 62·2; H, 6·8, 6·8; Cl, 8·7. Calc. for $C_{21}H_{26}O_4N_2$,HCl : C, 62·0; H, 6·7; Cl, 8·7%).

These analyses indicate that the new base contains $-CH_2$ less than echitamine, and determinations showed that it still contained a methylimino-group but had lost the methoxyl group of echitamine (Found for air-dry base : MeO, 0; NMe, 7·1. $C_{21}H_{26}O_4N_2$ requires NMe, 7·8%. Found for dry hydrochloride : MeO, 0; NMe, 6·8. $C_{21}H_{26}O_4N_2$,HCl requires NMe, 7·1%). The new base is therefore called *demethylechitamine* and all its reactions indicate that the methyl group has been lost from a carbomethoxy-group. This assumption accounts for the neutrality to litmus of demethylechitamine and the acidity to litmus of the hydrochloride, echitamine being strongly alkaline and its hydrochloride neutral to this indicator. The new base, like echitamine and all its salts, gives an intense blue coloration with Hopkin and Cole's glyoxylic reagent for tryptophan.

Action of Nitric Acid on Echitamine.—Harnack stated (*Ber.*, 1878, **11**, 2004) that a carmine-red base was produced by the action of concentrated nitric acid on echitamine, but the substance was not analysed or further described. When nitric acid (18 c.c.) is added to echitamine nitrate (3·19 g.) in water (180 c.c.) and the solution is warmed at 100°, it becomes red, then green, and finally yellow. When sodium hydroxide solution is added to the cooled liquid, a red base is precipitated; and a little more can be recovered by extraction with chloroform. A solution of the red base in dilute alcohol on concentration and standing deposits rosettes of minute needles (yield 1·4 g.), but the bulk of it remains amorphous. After recrystallisation the air-dry base began to decompose at 156° and after drying at 105° in a vacuum showed shrinkage at 163° (corr.) and frothed at 184° (corr.). It is almost insoluble in water, forms a red solution in alcohol and in chloroform a yellow solution with a red fluorescence [Found in air-dry base : loss at 105° in a vacuum, 12·1. $C_{22}H_{26}O_4N_2(NO_2)_2,4H_2O$ requires H_2O, 13·2%. Found in base dried at 105° in a vacuum : C, 55·6; H, 6·0; N, 11·6. Calc. : C, 55·7; H, 5·5; N, 11·8%].

The red base still contains intact the methoxyl group of echitamine (Found in dry base : MeO, 6·9. Calc., 6·5%).

The red colour is sharply changed to yellow on neutralisation with acid, and on adding standard alkali to a solution of a known weight of the base in a known excess of standard acid a sharp end-point is reached when a slight permanent red precipitate is formed. A molecular-weight determination by this method gave 474 [Calc. for $C_{22}H_{26}O_4N_2(NO_2)_2$, *M*, 489]. The red base appears therefore to be a simple dinitro-derivative of echitamine, but the change is

probably more far-reaching than is indicated by the empirical formula.

On treatment with sodium nitrite and dilute hydrochloric acid at 0°, echitamine gives a small yield (8%) of a substance crystallising from ether in rosettes of yellow needles, which shrinks at 140° and melts at 157° (decomp.) and, as it gives Liebermann's reaction, is probably a nitroso-derivative.

Distillation of Echitamine with Alkalis.—When echitamine is heated dry with soda-lime or with an aqueous solution of potassium hydroxide (50%), an alkaline, aqueous distillate is obtained when the temperature is kept below 250°. On neutralisation with hydrochloric acid and redistillation, this affords a distillate which when poured on a hot, oxidised copper spiral yields formaldehyde, due to the presence of methyl alcohol liberated by the hydrolysis of the carbomethoxy-group already referred to (p. 1646). The aqueous liquor in the distillation flask, on evaporation to dryness, left a small quantity of a hydrochloride.

The distillation was continued up to 310° and finally under reduced pressure until nothing more came over. The distillates thus obtained were acidified and extracted with ether, which removed 0·68 g. (from 8·6 g. of alkaloid) of an oil, having a pronounced fæcal odour. This oil gave a red coloration with Ehrlich's reagent, an orange colour with Nelson's solution, and dyed a pine splint, moistened with hydrochloric acid, red. It appeared therefore to be an indole derivative, but no well-defined product could be obtained from it. The acid mother-liquor was rendered alkaline and again extracted with ether, which removed 0·3 g. of a base from which also no crystalline derivatives could be obtained. The alkaline mother-liquor was then distilled, the distillate collected in dilute hydrochloric acid, and the salt produced isolated by the addition of alcohol to the highly concentrated liquor. The crystalline hydrochloride so obtained contained 52·5% of chlorine (Calc. for $CH_3{\cdot}NH_2,HCl$: Cl,52·5%).

The products of this distillation are therefore methyl alcohol, methylamine, an indole derivative, and a third base.

Non-alkaloidal Constituents.

Isolation of a Lactone, $C_9H_{14}O_3$.—No attempt has been made to isolate the components of the waxy and other materials removed in extracts A and B (p. 1642) in the purification of the acetic acid solution, but as these materials retained some alkaloid they were extracted with dilute hydrochloric acid, which was then rendered alkaline with ammonia and re-extracted with chloroform. In this way a crystalline, nitrogen-free product was isolated, more of which

was obtained from the mother-liquors remaining from the separation of crude echitamine hydrochloride. The total yield amounted to 0·02% by weight of the bark used. No evidence has been obtained that this substance is a decomposition product of echitamine. It crystallises from hot water in colourless prisms or long needles, m. p. 74—77° (air-dry; corr.) or 103° (dry; corr.), and has $[\alpha]_D^{15^\circ} + 49{\cdot}9^\circ$ (air-dry substance in water, $c = 2{\cdot}5$) or $+ 56{\cdot}4^\circ$ (dry substance in water, $c = 2{\cdot}5$), is neutral in reaction, fairly soluble in water or chloroform and sparingly soluble in ether (Found : loss on drying in a vacuum at 60° and finally at 90°, 9·84. $C_9H_{14}O_3,H_2O$ requires H_2O, 9·57%. Found in dried substance : C, 63·4, 63·7; H, 8·4, 8·5. $C_9H_{14}O_3$ requires C, 63·5; H, 8·3%).

No methoxyl or dioxymethylene group is present. The substance dissolves in solutions of sodium hydroxide, and the excess of alkali can be titrated back by standard hydrochloric acid with phenolphthalein as indicator. Such determinations indicated that the hydrated substance neutralised 20·7% of sodium hydroxide (Calc., 21·3%) and the dry substance, 23·5% (Calc., 23·5%). A solution in the calculated quantity of sodium hydroxide solution was concentrated, and kept after the addition of dry alcohol, when the corresponding *sodium* salt was obtained as a hygroscopic, crystalline substance (Found : loss on drying at 90—100° in a vacuum, 15·99. Calc. for $2H_2O$, 14·63%. Found in dry salt : Na, 10·64. Calc. for $C_9H_{15}O_4Na$: Na, 10·94%). The substance is therefore a *lactone* of an acid $C_9H_{16}O_4$.

On boiling the lactone with five times its weight of acetic anhydride for 1 hour, concentrating the solution under reduced pressure to half its volume, pouring into water, and extracting with ether we obtained a *monoacetyl* derivative which could not be crystallised (Found for substance dried in a vacuum at 105° : C, 62·5; H, 7·8. $C_9H_{13}O_3Ac$ requires C, 62·2; H, 7·6%). On hydrolysis with alkali and distillation of the resulting liquid, after addition of excess of sulphuric acid, 32·1% of acetic acid was obtained as determined by titration (acetic acid required for $C_9H_{13}O_3Ac$, 28·3%). The residue in the flask, on thorough extraction with chloroform, yielded 79·3% by weight of the original substance used (Calc. for $C_9H_{14}O_3$ from $C_9H_{13}O_3Ac$, 80·2%). The recovered lactone had m. p. 98—100° and $[\alpha]_D^{15^\circ} + 55{\cdot}8^\circ$ after drying in a vacuum at 100°.

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